

Original Article

Prevalence and antibacterial resistance patterns of uropathogenic staphylococci in Casablanca, Morocco

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Abstract

Introduction: The purpose of this research is to evaluate the resistance profile of uropathogenic staphylococci bacteria in Casablanca, Morocco. **Methodology:** In this retrospective cross-sectional research carried out from January 2017 to December 2020, isolation and identification were carried out according to the usual techniques in medical microbiology. *Staphylococcus aureus* isolates were confirmed by polymerase chain reaction (PCR) amplification of the *nuc* gene, and the antibiogram was performed according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM 2021). The susceptibility of uropathogenic staphylococci to vancomycin was determined with broth microdilution following the recommendations of the Clinical and Laboratory Standards Institute. The *mecA* gene was tested on phenotypically cefoxitin-resistant *S. aureus* isolates by PCR.

Results: The prevalence of urinary tract infections (UTIs) was 18% (772/4374). UTIs were more common in females (n = 483, 63%) than males (n = 289, 37%). Among the Gram-positive bacteria isolated (198, 25.65%), the prevalence of staphylococci was (130/198, 65.66%). Among staphylococcal species identified, coagulase-negative staphylococci (CoNS) were more prevalent (112/130, 86.15%), and *Staphylococcus saprophyticus* was the most frequently isolated CoNS (46/112, 41.07%). Additionally, there were several *S. aureus* strains (18/130, 13.85%). Forty-four percent of *S. aureus* isolates (n = 8) were resistant to cefoxitin and also harboured the *mecA* gene. All *S. aureus* isolates were susceptible to linezolid, cotrimoxazole and vancomycin.

Conclusions: The prevalence and antibacterial resistance patterns of uropathogenic staphylococci in this study, with a high percentage of methicillin resistance, require careful consideration of antimicrobial therapy for staphylococcal UTIs.

Key words: UTIs; *S. aureus*; CoNS; antibiotics; MRSA.

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Introduction

Urinary tract infections (UTIs) are the second most frequent infectious diseases in hospitals and in the community, after respiratory tract infections [1]. Hence, there are high morbidity and mortality rates and high economic costs associated with their treatment and health care [2]. The average global incidence of UTIs is estimated to be between 150 and 250 million cases per year [3], which causes an expenditure of more than 6 billion dollars in health care spending for treatment and hospitalization [4].

UTIs are usually characterized by the appearance of significant symptomatic bacteriuria in the urinary tract [5]. They can be caused by a wide variety of microorganisms, although members of the

Enterobacteriaceae family and in particular, *Escherichia coli* are the most frequently involved [6], because they belong to the human microbiota and easily colonize the urinary tract [7].

In recent years, *Staphylococcus* species have developed a variety of virulence factors that maintain their pathogenicity and high affinity for urinary tract epithelial cells. These factors have contributed to a more complete understanding of their pathogenic role in UTIs, particularly in the elderly, pregnant women or those with other risk factors for developing a UTIs [8]. The presence or absence of coagulase has traditionally been used to classify staphylococcal species into coagulase-negative staphylococci (CoNS) or coagulase-positive *Staphylococcus aureus* [9]. The

second leading cause of UTIs in sexually active young females is *Staphylococcus saprophyticus*, which is characterized phenotypically by its resistance to novobiocin [10]. Other CoNS isolated from UTIs, such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*, have not frequently been identified at the species level; therefore, their functions in UTIs could not be determined [11].

The treatment of UTIs in inpatient and outpatient settings is becoming increasingly difficult because of the increase in antibiotic resistance of these bacteria [12] due to excessive and inappropriate use of antibiotics and the selection of resistant mutant bacteria, leaving clinicians with limited therapeutic alternatives [13]. UTI epidemiology and antibiotic resistance trends show significant geographical and temporal heterogeneity. As a result, utilizing analytical epidemiology to examine these data is crucial to reflect on the national condition in comparison to worldwide statistics [14]. Knowing the antibiotic resistance tendencies might help clinicians to choose the best antibiotic treatments for their patients [12].

The term methicillin-resistant *Staphylococcus* corresponds to strains of *Staphylococcus* that can resist antibiotics including methicillin, cephalosporins, oxacillin, imipenem and other β -lactam antibiotics [15]. Pathogenic staphylococci are well known for their resistance and clinical importance. In addition, the slow but notable emergence of intermediate- and vancomycin-resistant staphylococci in UTIs should be mentioned as a formidable obstacle to treatment [8]. The focus of this research was to retrospectively evaluate 4 years of antibiotic resistance trends and the epidemiology of *Staphylococcus* species.

Methodology

Research area

Greater Casablanca is the most densely populated region in Morocco. It comprises seven cities (Berrechid, Ben Slimane, Casablanca, Mohammedia, El Jadida, Sidi Bennour and Settat). Greater Casablanca draws a considerable number of migrants from all across Morocco, including the countryside, resulting in considerable socio-economic heterogeneity [16]. Therefore, the population of this region can be considered reasonably indicative of the Moroccan population.

Study design and population

This research was designed as a cross-sectional survey of uropathogenic staphylococci isolated from

patients with UTIs between 1 January 2017 and 30 December 2020. Overall, 4374 urine samples were collected in the bacteriology laboratory of the Pasteur Institute of Morocco, Casablanca (879 samples), and in 20 other private medical laboratories in greater Casablanca: 15 laboratories in the northern part of the region (Casablanca, Mohammedia and Ben Slimane; 2895 samples), two laboratories in the central part of the region (Berrechid and Settat; 298 samples), and three laboratories in the southern part of the region (El Jadida and Sidi Bennour; 302 samples). Mid-stream clean catch urine was collected according to standard procedures in the medical clinical laboratory.

Variables

The date of collection, age, gender, urine culture findings, identification of the bacterial strain causing the UTIs, and the associated antibiotics resistance bacteria findings were all recorded for each patient.

Collection, identification and conservation of strains

According to the medical microbiology reference (REMIC 2018) [17], the biological inclusion criterion for this study was a pure bacterial culture with a colony count of $> 1 \times 10^5$ colony-forming unit (CFU)/mL and a leucocyte count of $> 1 \times 10^4$ /mL.

Cystine lactose electrolyte deficient (CLED) agar (Biokar Diagnostics, Beauvais, France) and UriSelect chromogenic agar (Bio-Rad, Hercules, CA, USA) were used to inoculate 10 μ L of urine according to the usual techniques in medical microbiology. After incubation at 35 ± 2 °C aerobically overnight, urine cultures were classified as negative when bacterial growth was $< 1 \times 10^3$ CFU/mL or positive when monomorphic bacterial growth was $> 1 \times 10^5$ CFU/mL and the patient exhibited UTI symptoms (inclusion criteria). For these cases, clinical isolates of *Staphylococcus* species were identified using medical microbiological techniques, including biochemical reactions: Gram staining, catalase and coagulase activity and mannitol salt agar (Biokar Diagnostics, Beauvais, France) fermentation. The VITEK 2[®] COMPACT 15 system (bioMérieux, Marcy-l'Étoile, France) was used for identification according to standard criteria.

The isolates were stored in brain heart infusion (BHI) broth (Biokar Diagnostics, Beauvais, France) with 15% glycerol at -80 °C until use.

The reference strains *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were used as positive controls.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion technique on Mueller–Hinton agar medium (Bio-Rad, Marnes-la-Coquette, France) and the automated VITEK 2[®] COMPACT 15 system (bioMérieux, Marcy-l'Étoile, France).

The inhibition zone diameters were measured after an overnight incubation. Each strain was categorised as susceptible (S), sensitive at a high dosage (I) or resistant (R) following the Antibiogram Committee of the French Microbiology Society (CA-SFM 2021) guidelines [18]. The antibiotic disks used were penicillin-G (1 units), ceftiofur (30 µg), levofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), tigecycline (15 µg), linezolid (10 µg) and fusidic acid (10 µg).

MIC of vancomycin-resistant *Staphylococcus* species

Minimum inhibitory concentrations (MICs) were measured for resistant strains by using the liquid microdilution method with cation-adjusted BHI broth and the VITEK 2[®] COMPACT 15 system antibiogram. The procedure for this method was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. Seven vancomycin concentrations were tested: 16, 8, 4, 2, 1, 0.5 and 0.25 µg/mL. The findings were interpreted according to the CLSI guidelines [18]. A MIC ≤ 2 µg/mL was considered sensitive, a MIC between 4 and 8 µg/mL was considered intermediate and a MIC ≥ 16 µg/mL was considered resistant.

Inducible clindamycin resistance: D test

All *Staphylococcus* spp isolates with erythromycin resistance were tested for inducible resistance to clindamycin based on the D test on Muller–Hinton agar. Three distinct phenotypes of *S. aureus* strains were deduced from the results of this induction test.

- The moderate sensitive phenotypes (MS): *Staphylococcus* spp isolates are resistant to

erythromycin and sensitive to clindamycin but without a D-shaped zone.

- The inducible clindamycin resistance phenotype (iMLS_B): *Staphylococcus* spp isolates are resistant to erythromycin and sensitive to clindamycin and had a D-shaped zone.
- The constitutive clindamycin resistance phenotype (cMLS_B): *Staphylococcus* spp strains are resistant to both clindamycin and erythromycin.

Detection of methicillin resistance

Phenotypic resistance to methicillin was tested using a ceftiofur disk (30 µg) under standard susceptibility testing conditions and by plating on Mueller–Hinton agar, incubating at 35 °C ± 2 for 24 ± 4 h and measuring the zone of inhibition in millimetres [18]. It was categorised as resistant if the zone of inhibition was > 22 mm in diameter and sensitive if it was < 22 mm in diameter [18].

S. aureus ATCC 43300 was used as a positive control and *S. aureus* ATCC 25923 was used as a negative control.

Molecular confirmation of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) isolates

Molecular confirmation was performed by amplification of the *nuc* gene to identify positive *S. aureus* isolates. Polymerase chain reaction (PCR) identification of the *mecA* gene was performed in isolates with ceftiofur-resistant phenotype.

DNA extraction

All *Staphylococcus* spp isolates were cultivated on mannitol salt agar at 37 °C overnight. Genomic DNA was extracted from pure cultures using the InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions and the purified DNA was used as a template for PCR. This DNA was stored at -20 °C until use.

Table 1. Target genes, their primer pairs, and cycling conditions for the *nuc* and *mecA* genes used in this study.

Target gene	<i>nuc</i>	<i>mecA</i>
Primer pairs	5'-GCGATTGATGGTGATACGGTT-3' 5'-AGCCAAGCCTTGACGAATA AAGC-3'	5'-GATATCGAGGCCCGTGGATT-3' 3'-ACGTGCAACTTGAGCTGTTA-5'
PCR product	270 bp	642 bp
Cycling conditions	Initial denaturation at 94 °C for 5 min. (35 cycles): - Denaturation at 94 °C for 30 s. - Annealing at 55 °C for 30 s. - Polymerization at 72 °C for 1 min. - Final extension step at 72 °C for 10 min [20].	Initial denaturation at 95 °C for 1 min. (35 cycles): - Denaturation at 94 °C for 1min. - Annealing at 52 °C for 1min. - Polymerization at 72 °C for 1min. - Final extension step at 72 °C for 10 min [21].

PCR amplification

PCR reaction was performed using 2 µL of the DNA template, 10 µL of 5x MyTaq Reaction Buffer (Bioline Reagents, London, UK) containing 5 mM of dNTPs, 15 mM of MgCl₂, stabilisers and enhancers at optimal concentrations (eliminating the need for optimisation), 1 µL of MyTaq DNA Polymerase, 1 µL of each primer at a concentration of 20 pmol, and double-distilled water. The primer pairs and cycling conditions used in the PCR are summarized in Table 1 [20-22].

After running the PCR, 10 µL of the product was separated by electrophoresis in a 1.5% agarose gel (Sigma, Darmstadt, Germany) stained with 0.5 µg/mL ethidium bromide (Sigma, Darmstadt, Germany) in 1× TAE buffer for 30 min and then visualised under UV Light.

Statistical analysis

The data were analysed using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). The date of isolation, demographic characteristics, the isolate detected, and the antibiotic susceptibility patterns were evaluated. The Chi-square test was calculated to ascertain the association between patient demographics and UTIs. A *p* value ≤ 0.05 was considered to be statistically significantly.

Ethical consideration

This study received approval from the Internal Ethics Committee of the Department of Biology-Geology, Polydisciplinary Faculty of Sultan Moulay Slimane University, Beni Mellal.

Results

Table 2. Prevalence of uropathogenic bacteria.

Bacteria		%	
Enterobacteriaceae (n = 520)			
	<i>Escherichia coli</i>	75.38 (392/520)	
	<i>Klebsiella</i> spp	16.54 (86/520)	
	<i>Enterobacter</i> spp	5.00 (26/520)	
	<i>Proteus</i> spp	3.08 (16/520)	
Non fermenting Enterobacteriaceae (n=45)		5.83 (45/772)	
Gram positive (n = 198).	<i>Staphylococcus</i> spp (n = 130) CoNS (n = 112)	<i>S. auriculari</i>	2.68 (3/112)
		<i>S. epidermidis</i>	28.57 (32/112)
		<i>S. haemolyticus</i>	12.50 (14/112)
		<i>S. Hominis</i>	7.14 (8/112)
		<i>S. saprophyticus</i>	41.07(46/112)
		<i>S. Simulans</i>	2.68 (3/112)
		<i>S. warneri</i>	5.36 (6 /11)
		<i>S. aureus</i> (n = 18)	13.85 (18/130)
		<i>Streptococcus</i> spp (n = 20)	10.09 (3/112)
		<i>Enterococcus</i> spp (n = 48)	48.62 (3/112)
<i>Candida</i> spp (n = 9)		1.17	

CoNS: coagulase-negative *Staphylococcus*.

Characteristics of patients

A total of 4,374 urine samples were collected during the research period, of which 772 were positive urine cultures, representing UTIs prevalence rate of 18%. Fever, burning on urination and kidney problems were among the most common symptoms in those with UTIs.

The UTIs were significantly associated with the female gender, with a male to female gender ratio of 0.59 (483 females [63%] versus 289 males [37%], *p* = 0.029). The mean ± standard deviation age of the infected patients was 34.77 ± 31.61 years (range 1 month to 97 years). People of both genders and of all ages are susceptible to UTIs. The prevalence of those infections was higher in the age groups of 0–14 years, 25–64 years and > 67 years, with 36.92%, 30.05% and 29.66%, respectively. The age group of 15–24 years had the lowest prevalence of 3.37%.

Infection prevalence and pathogens

Of the 772 isolates, 520 (67.36%) were members of Enterobacteriaceae, 45 (5.83%) were Gram-negative non-fermenting bacteria, 198 (25.65%) were Gram-positive bacteria and 1.17% were *Candida* spp (Table 2).

Gram-negative bacilli were the most predominant uropathogenic bacteria, with *E. coli* accounting for 75.38% (n = 392) and *Klebsiella* spp accounting for 16.54% (n = 86) of the isolates (Table 2). Nevertheless, Gram-positive bacteria were a common cause of UTIs, especially in the elderly, pregnant women and people with other risk factors for UTIs. Among the Gram-positive bacteria isolated (n = 198), *Staphylococcus* spp (130/198, 65.66%) was the most frequent, followed by *Enterococcus* spp (48/198, 24.25%) and *Streptococcus* spp (20/198, 10.09%) (Table 2).

Regarding the staphylococcal species identified, CoNS were more prevalent, at 86.15% (112/130). On the other hand, *S. aureus* was identified in a minority of cases (18/130, 13.85%); these isolates tested positive for the *nuc* gene. *S. saprophyticus* was the most frequently isolated CoNS (46/112, 41.07%), followed by *S. epidermidis* (32/112, 28.57%) and *S. haemolyticus* (14/112, 12.5%) (Table 2).

Antibiotics resistance profile of uropathogenic staphylococci isolates

The antibiotic resistance profiles of uropathogenic *Staphylococcus* spp were determined by following the recommendations of CA-SFM 2021 [18].

β -lactam antibiotic resistance profile

In this study, *S. saprophyticus*, *S. aureus*, *S. epidermidis* and *S. haemolyticus* were the most frequently identified *Staphylococcus* species. They showed significant penicillin resistance: 100%, 83.33%, 81.25% and 64.28%, respectively. The disk diffusion method of cefoxitin on the Mueller–Hinton agar medium revealed that 44.44% *S. aureus* (n = 8) isolates were resistant to cefoxitin. On the other hand, a significantly higher proportion of CoNS isolates were resistant to cefoxitin, with 69% of *S. epidermidis* and 64.28% of *S. haemolyticus* exhibiting resistance to this antibiotic.

After confirmation of the phenotypic test for cefoxitin resistance, a genotypic PCR test was performed to identify the *mecA* gene. All cefoxitin-resistant *S. aureus* isolates were positive for the *mecA* gene, a finding consistent with the results of the PCR amplification test. All *mecA*-positive strains isolated in this study were sensitive to vancomycin, gentamicin and linezolid and resistant to multiple antibiotics (Table 3).

Aminoglycoside antibiotic resistance profile

All *S. aureus* and *S. saprophyticus* isolates were susceptible to aminoglycoside antibiotics (gentamicin, tobramycin and kanamycin). Sixty-four percent of *S. haemolyticus* isolates were resistant to kanamycin and tobramycin, and over 36% were resistant to gentamicin. In addition, 44 % of *S. epidermidis* strains were resistant to kanamycin, 34.3% of isolates were resistant to tobramycin and 12.5% of isolates were resistant to gentamicin. Sixty-three percent of *S. hominis* isolates were resistant to gentamicin, kanamycin and tobramycin. In contrast, gentamicin and tobramycin remained effective against *S. warneri* isolates (Table 4).

Macrolide antibiotic resistance profile

Forty-four percent of *S. aureus* isolates, 43.47% of *S. saprophyticus* isolates and 43.75% of *S. epidermidis* isolates were resistant to erythromycin. In contrast, *S. haemolyticus* and *S. hominis* isolates had the lowest resistance to erythromycin, with 35.7% and 37.5%, respectively (Table 4). Both erythromycin and clindamycin were effective against 59.23% of *Staphylococcus* spp. On the other hand, 23.85% of *Staphylococcus* spp had the cMLSB phenotype, 6.92% had the MS phenotype and 10 % had the iMLSB phenotype.

Fluoroquinolone antibiotic resistance profile

All examined *S. aureus*, *S. saprophyticus*, *S. hominis* and *S. warneri* strains were sensitive to fluoroquinolones. Although *S. epidermidis* and *S. haemolyticus* exhibited 40.63% and 35.71% resistance to levofloxacin, respectively, these strains are also resistant to all other fluoroquinolone antibiotics (Table 4).

Table 3. Antibiotic resistance profile of *S. aureus* isolates.

Antibiotics	<i>S. aureus</i> (%)		<i>p</i> value
	<i>MecA</i> + (44.4%)	<i>MecA</i> - (55.6%)	
Penicillin G	100	70	1
Tobramycin	0	0	-
Gentamycin	0	0	-
Erythromycin	62.5	10	0.48
Clindamycin	12.5	0	0.42
Tetracycline	62.5	10	0.48
Levofloxacin	0	0	-
Linezolid	0	0	-
Fusidic Acid	100	10	0.14
Vancomycin	0	0	-
Teicoplanin	0	0	-
Trimethoprim-sulfamethoxazole (SXT)	100	0	-

Linezolid, cotrimoxazole, vancomycin and fusidic acid antibiotic resistance profiles

The most effective antibiotics against *S. aureus* isolates were linezolid, cotrimoxazole and vancomycin (100%). The vancomycin MICs for the various *S. aureus* strains ranged from 0.5 to 1 µg/mL. *S. saprophyticus* isolates were highly resistant to fusidic acid (60.87%) and were least resistant to linezolid (19.57%), cotrimoxazole (20%) and vancomycin (10.87%). *S. epidermidis* showed the highest resistance to fusidic acid (50%). On the other hand, there was very low resistance to linezolid, cotrimoxazole and vancomycin. The vancomycin MICs for the *S. epidermidis* and *S. saprophyticus* strains ranged from 0.5 to more than 16 µg/mL. Linezolid, cotrimoxazole and vancomycin were highly effective against all strains of *S. haemolyticus*, *S. hominis* and *S. warneri*. In contrast, these strains exhibited high resistance to fusidic acid (64.28%, 62.5% and 50%, respectively) (Table 4).

Discussion

UTIs are among the most widespread bacterial infections in the community and hospital settings, particularly within the health care context. Throughout the world, UTIs impact patients of all ages and both genders. Compared to previous research [23-25], the prevalence of UTIs in this study was very low (18%), most likely due to demographic and sample size differences. Additionally, the advancements in UTI treatment [26] and public hygiene [27], over time may have reduced the prevalence of UTIs. According to literature, UTIs are more prevalent in women than in men. In the current study, 63% of those infected were women while 37% were men. Indeed, UTIs in women may be promoted by various factors related to the anatomical and physiological characteristics of their urinary tract and hormonal variation [24,28].

E. coli was the most isolated bacterium in the samples (75.38%), followed by *Klebsiella* spp strains (16.54%). These results are consistent with several studies [29,30]. UTIs are much less commonly caused by *Staphylococcus* spp. Of the isolated CoNS, 41.07% were *S. saprophyticus*, 12.50 % were *S. haemolyticus* and 25.89% were *S. epidermidis*. These results are comparable to previous studies conducted at the University Hospital in Tahar Sfar, Mahdia, Tunisia [31], and in the Nemba District Hospital in Rwanda [32].

Colonisation of the gastrointestinal tract by *S. saprophyticus* has been associated with UTIs, with a pathophysiology that is similar to UTIs caused by *E. coli* [32].

Ingestion of contaminated food products, which can promote colonisation and subsequent UTIs, accounted for the high isolation rate of *S. saprophyticus* strains in the current study [33]. In addition to nutrition, other environmental and human causes of *S. saprophyticus* infection include the marine environment [34], genitourinary abnormalities [35], recent sexual activity [36], and previous exposure to raw meat or antibiotics [37]. The relationship between outdoor swimming and *S. saprophyticus* colonization has been clearly documented [35].

The prevalence rate of *S. aureus* causing UTIs in this study (13.85%) is lower than in similar studies conducted in the Aljouf region of northern Saudi Arabia [38] and in the northern province of Rwanda in the Gakenke district [28]. The clinical significance of *S. aureus* isolation in the urine is undetermined. Furthermore, several factors can explain the isolation rate of *S. aureus* in this study, such as urinary tract instrumentation, long-term care, urological surgical procedures, urinary tract obstruction, older age, hospital exposure, malignancy and the presence of an indwelling catheter [39].

Table 4. Level of antimicrobial resistance of CoNS isolates (%).

Antibiotics	CoNS isolates (%)				
	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. warneri</i>
Penicillin G	100	81.25	64.28	100	100
Kanamycin	0	43.75	64.28	62.5	50
Tobramycin	0	34.37	64.28	62.5	0
Gentamycin	0	12.50	35.71	62.5	0
Erythromycin	43.47	43.75	35.71	37.5	50
Tetracycline	10.87	68.75	64.28	37.5	0
Levofloxacin	0	40.63	35.71	0	0
Linezolid	19.57	9.38	0	0	0
Fusidic Acid	60.87	50	64.28	62.5	50
Vancomycin	10.87	9.38	0	0	0
Teicoplanin	0	18.75	0	0	0

CoNS: coagulase-negative *Staphylococcus*.

Staphylococcal bacteraemia and invasive *S. aureus* pathologies such as renal abscesses, pyomyositis, osteomyelitis, endocarditis, thrombophlebitis, and septic arthritis can be caused by *S. aureus* UTIs [40]. The causes and relationships between persistent *S. aureus* UTIs and the percentage of patients who develop bacteraemia are unclear [39]. Recent research has shown that chronic *S. aureus*-associated bacteriuria may be a sign of severe *S. aureus* bacteraemia and invasive *S. aureus* infections [40]. In addition, *S. aureus* UTIs are seen in approximately 7–16% of patients with *S. aureus* bacteraemia, particularly in cases of endocarditis, and are considered an indicator of haematogenous seeding of the renal parenchyma [41].

MRSA has become a global public health challenge and is known as a serious pathogenic bacterium that can cause community- and hospital-acquired infections with high morbidity and mortality despite the use of antibiotics [15]. In addition, in patients with hospital-acquired MRSA, the urinary tract is a frequent site of colonisation and infection [15]. Furthermore, MRSA makes it problematic to determine the specific function of *S. aureus* as a causative factor in symptomatic UTIs [15]. In this study, the prevalence of *S. aureus* was 7.32%, and MRSA accounted for 43% of *S. aureus* isolates. This prevalence is higher than that reported in studies conducted in University Hospital Waterford, Waterford, Ireland [42], in the Arba Minch General Hospital, Southern Ethiopia [15], and in Prince Mutaib Hospital, Sakaka, Aljouf, Saudi Arabia [38].

The aetiology of staphylococci that cause UTIs as well as their antimicrobial resistance varies over time and differs among countries, ranging from high to moderate to mild. In this study, the most frequently isolated *Staphylococcus* spp uropathogens were all quite resistant to penicillin: 100%, 83.33%, 81.25% and 64.28% for *S. saprophyticus*, *S. aureus*, *S. epidermidis* and *S. haemolyticus*, respectively. These resistance rates are consistent with the results of other studies [15,43]. This global state of β -lactam antibiotic resistance is the result of selection pressure caused by the inappropriate and intensive use of β -lactam antibiotics in health care facilities and in self-medication.

Aminoglycosides are a type of antibiotic that can be used in combination with glycopeptides or β -lactams to treat complicated staphylococcal UTIs [44]. Aminoglycosides bind to the 16S subunit of bacterial ribosomal RNA, interfering with protein biosynthesis and ultimately leading to cell death [45]. All *S. aureus* and *S. saprophyticus* isolates in the current study were completely susceptible to aminoglycoside antibiotics.

Because aminoglycoside antibiotics are effective against *Staphylococcus* species, rational use of these molecules is necessary to prevent the emergence of multidrug-resistant strains. However, 64.28% of *S. haemolyticus* isolates were resistant to kanamycin and tobramycin, and 35.71% of the isolates were resistant to gentamycin. This result is comparable to that reported by Haque *et al.* [46] in 2021 in Bangladesh. On the other hand, 43.75% of *S. epidermidis* isolates were resistant to kanamycin, while 34.37% were resistant to tobramycin. These results contrast with those published by Anam *et al.* [47] in 2015 in Pakistan, with gentamicin resistance of 92.3%.

The macrolide family, including erythromycin, is widely used to treat UTIs caused by a variety of bacteria, including uropathogenic *Staphylococcus* [48]. Erythromycin inhibits the synthesis of proteins necessary for bacterial activity, which reduces bacterial growth and prevents the peptide chain from leaving the bacterial ribosome by binding to the 23S ribosomal RNA molecule in the 50S subunit [49]. Forty-four percent of *S. aureus* isolates, 43.47% of *S. saprophyticus* isolates and 43.75% of *S. epidermidis* isolates were resistant to erythromycin. This finding is similar to that found by Simon-Oke *et al.* [50] in 2019 in Akure, Nigeria, and by Ulrika Windahl *et al.* [51] in 2014 in Uppsala, Sweden. In this study, *S. haemolyticus* and *S. hominis* isolates showed the lowest resistance to erythromycin (35.71% and 37.5%, respectively). These resistance rates are lower than reports from Rajshahi, Bangladesh [46], Rio de Janeiro, Brazil [50], and Akure, Nigeria [43], which reported that 76.93%, 64% and 50% of *S. haemolyticus* strains, respectively, were resistant to erythromycin.

UTIs are most often treated with fluoroquinolones such as levofloxacin and ciprofloxacin, which are broad-spectrum antibiotics against both Gram-positive and Gram-negative bacteria [23]. Levofloxacin-resistant *Staphylococcus* species have emerged worldwide due to the increased use of fluoroquinolones [52]. In this study, fluoroquinolone antibiotics were more effective against *S. aureus*, *S. saprophyticus*, *S. hominis* and *S. warneri*. This finding is consistent with other studies that have shown high sensitivity to fluoroquinolones [50]. The isolates showed the lowest resistance to levofloxacin: 40.63% for *S. epidermidis* and 35.71% for *S. haemolyticus*. There have been reports of higher resistance of *S. haemolyticus* to ciprofloxacin: 50% in a study on uropathogenic bacteria in Jakarta, Indonesia [53], and 73.08% in a study conducted to evaluate the antibiogram profiling of

multidrug resistant *S. haemolyticus* isolated from patients with UTIs in Bangladesh [46].

In the present study, all uropathogenic *Staphylococcus* spp isolates were susceptible to linezolid, cotrimoxazole and vancomycin. This result is comparable to other studies conducted worldwide [54]. Linezolid resistance in uropathogenic staphylococci is rare, while prolonged exposure to vancomycin results in the emergence of staphylococci with reduced susceptibility to vancomycin, and the strains are classified as having intermediate vancomycin resistance.

Conclusions

To the best of our knowledge, this is the first extensive research on the prevalence and antibiotic resistance of staphylococcal strains isolated from urine in Morocco. Among the Gram-positive bacteria isolated, the prevalence of uropathogenic staphylococci was 65.66%, with *S. aureus* isolates representing 13.85%. Eight *S. aureus* isolates were MRSA strains (8/18, 44.4%). Almost all *Staphylococcus* spp isolates were susceptible to linezolid, cotrimoxazole and vancomycin; however, they showed high resistance to penicillin G, fusidic acid and kanamycin. These findings emphasise the need for continuous antimicrobial resistance surveillance and for special precautions when designing empirical treatment.

References

- Arjunan M, Al-Salamah AA, Amuthan M (2010) Prevalence and antibiotics susceptibility of uropathogens in patients from a rural environment, Tamil Nadu. *Am J Infect Dis* 6: 29-33. doi: 10.3844/ajidsp.2010.29.33.
- Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W (2001) Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother* 47: 773-780. doi: 10.1093/jac/47.6.773.
- Hrbacek J, Cermak P, Zachoval R (2020) Current antibiotic resistance trends of uropathogens in Central Europe: survey from a tertiary hospital urology department 2011-2019. *Antibiotics* 9: 630. doi: 10.3390/antibiotics9090630.
- Gebremariam G, Legese H, Woldu Y, Araya T, Hagos K, Gerbreyesus Wasihum A (2019) Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia. *BMC Infect Dis* 19: 950. doi: 10.1186/s12879-019-4610-2.
- Jhora ST, Paul S (2011) Urinary tract infections caused by *Staphylococcus saprophyticus* and their antimicrobial sensitivity pattern in young adult women. *Bangladesh Journal of Medical Microbiology* 5: 21-25. doi: 10.3329/bjmm.v5i1.15817.
- Dougnon V, Assogba P, Anago E, Déguénon E, Dapuliga C, Agbankpè J (2020) Enterobacteria responsible for urinary infections: a review about pathogenicity, virulence factors and epidemiology. *J App Biol Biotech* 8: 1-2. doi: 10.7324/JABB.2020.80118.
- Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 13: 269-284. doi: 10.1038/nrmicro3432.
- Gajdác M, Ábrók M, Lázár A, Burián K (2020) Increasing relevance of Gram-positive cocci in urinary tract infections: a 10-year analysis of their prevalence and resistance trends. *Sci Rep* 10: 17658. doi: 10.1038/s41598-020-74834-y.
- Foster T (1996) *Staphylococcus*. In Baron S, editor. *Medical Microbiology*, 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston. Available: <http://www.ncbi.nlm.nih.gov/books/NBK8448/>. Accessed: 18 February 2022.
- Ehlers S, Merrill SA (2022) *Staphylococcus saprophyticus*. *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available: <http://www.ncbi.nlm.nih.gov/books/NBK482367/>. Accessed: 18 February 2022.
- Natsis NE, Cohen PR (2018) Coagulase-negative *Staphylococcus* skin and soft tissue infections. *Am J Clin Dermatol* 19: 671-677. doi: 10.1007/s40257-018-0362-9.
- Issakhanian L, Behzadi P (2019) Antimicrobial agents and urinary tract infections. *Curr Pharm Des* 25: 1409-1423. doi: 10.2174/1381612825999190619130216.
- Arias CA, Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol* 10: 266-278. doi: 10.1038/nrmicro2761.
- Sader HS, Farrell DJ, Flamm RK, Jones RN (2014) Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalised with pneumonia in US and European hospitals: results from the SENTRY Antimicrobial Surveillance Program, 2009-2012. *Int J Antimicrob Agents* 43: 328-334. doi: 10.1016/j.ijantimicag.2014.01.007.
- Mitiku A, Aklilu A, Biresaw G, Gize A (2021) Prevalence and associated factors of methicillin resistance *Staphylococcus aureus* (MRSA) among urinary tract infection suspected patients attending at Arba Minch General Hospital, Southern Ethiopia. *IDR* 14: 2133-2142. doi: 10.2147/IDR.S306648.
- Institutional website of the Haut-Commissariat au Plan of the Kingdom of Morocco (n.d.). Morocco in figures. Available: https://www.hcp.ma/downloads/Maroc-en-chiffres_t13053.html. Accessed 18 February 2022. [Article in French].
- SFM (2018) Medical microbiology reference (REMIC 6.2), 6th ed. French Microbiology Society. 477-965. Available: <https://www.sfm-microbiologie.org/boutique/referentiel-en-microbiologie-medicale-remic/>. [Article in French].
- SFM (2021) Antibigram Committee of the French Microbiology Society. Available: https://www.sfm-microbiologie.org/wp-content/uploads/2021/04/CASFM2021_VI.0.AVRIL_2021.pdf? Accessed: 18 February 2022. [Article in French].
- Clinical and Laboratory Standards Institute (2019) VET01: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Available: <https://clsi.org/standards/products/veterinary-medicine/documents/vet01/>. Accessed: 3 January 2023.
- Stańkowska M, Garbacz K, Piechowicz L, Bronk M (2019) Dissemination of t437-SCCIV and coagulase-negative t037-SCC III types among borderline oxacillin-resistant *Staphylococcus aureus* isolated from skin infections and

- diabetic foot ulcers. *IDR* 12: 3197-3203. doi: 10.2147/IDR.S219557.
21. Al-Amery K, Elhariri M, Elsayed A, El-Moghazy G, Elhelw R, El-Mahallawy H, El Hariri M, Hamza D (2019) Vancomycin-resistant *Staphylococcus aureus* isolated from camel meat and slaughterhouse workers in Egypt. *Antimicrob Resist Infect Control* 8: 129. doi: 10.1186/s13756-019-0585-4.
 22. Zalegh I, Bourhia M, Zerouali K, Katfy K, Nayme K, Khallouki F, Benzaarate I, Mohammad Salamattullah A, Alzahrani A, Nafidi HA, Akssira M, Ait Mhand R (2022) Molecular characterization of gene-mediated resistance and susceptibility of ESKAPE clinical isolates to *Cistus monspeliensis* L. and *Cistus salvifolius* L. extracts. *Evid Based Complement Alternat Med* 2022: 7467279. doi: 10.1155/2022/7467279.
 23. Odoki M, Almustapha Aliero A, Tibyangye J, Nyabayo Maniga J, Wampande E, Drago Kato C, Agwu E, Bazira J. (2019) Prevalence of bacterial urinary tract infections and associated factors among patients attending hospitals in Bushenyi District, Uganda. *Int J Microbiol* 2019: 1-8. doi: 10.1155/2019/4246780.
 24. Allothman A, Thaqafi AA, Ansary AA, Zikri A, Fayed A, Khamis F, Al Salman J, Al Dabal L, Khalife N, AlMusawi T, Alfouzan W, El Zein S, Kotb R, Ghoneim Y, Kanj SS (2020) Prevalence of infections and antimicrobial use in the acute-care hospital setting in the Middle East: results from the first point-prevalence survey in the region. *Int J Infect Dis* 101: 249-258. doi: 10.1016/j.ijid.2020.09.1481.
 25. Mouanga Ndzime Y, Onanga R, Kassa Kassa RF, Bignoumba M, Mbehang Nguema PP, Gafou A, Lendamba RW, Mbombe Moghoa K, Bisseye C. (2021) Epidemiology of community origin *Escherichia coli* and *Klebsiella pneumoniae* uropathogenic strains resistant to antibiotics in Franceville, Gabon. *Infect Drug Resist* 14: 585-594. doi: 10.2147/IDR.S296054.
 26. Abedin M, Yeasmin F, Mia S, Helal H, Rasheda Y (2021) Bacteriological profile and antimicrobial susceptibility patterns of symptomatic urinary tract infection among patients of different age groups in a tertiary care hospital of Bangladesh. *Moroccan Journal of Biology* 17: 59-66.
 27. Odongo I, Ssemambo R, Kungu JM (2020) Prevalence of *Escherichia coli* and its antimicrobial susceptibility profiles among patients with UTI at Mulago Hospital, Kampala, Uganda. *Interdiscip Perspect Infect Dis* 2020: e8042540. doi: 10.1155/2020/8042540.
 28. Bosco MJ, Jonas B, Evelyne K, Pauline K (2020) Urinary tract infection and antimicrobial resistance profile in patients attending Nemba District Hospital in Rwanda. *Asian J Med Sci* 11: 101-105. doi: 10.3126/ajms.v11i6.29921.
 29. Hailaji NSM, Ould Salem ML, Ghaber SM (2016) Antibiotic susceptibility of uropathogenic bacteria in the city of Nouakchott - Mauritania. *Progress in Urology* 26: 346-352. [Article in French]. doi: 10.1016/j.purol.2016.04.004.
 30. Sekhsokh Y, Chadli M, El Hamzaoui SA (2008) Frequency and antibiotic susceptibility of bacteria isolated from urine. *Medicine and Infectious Diseases* 38: 324-327. [Article in French]. doi: 10.1016/j.medmal.2008.02.003.
 31. Ben Haj Khalifa A, Khedher M (2010) Frequency and antibiotic resistance of uropathogenic bacteria at Tahar Sfar University hospital in Mahdia. *Tunisian Journal of Infectiology* 4: 57-61. [Article in French].
 32. Tamura D, Yamane H, Tabakodani H, Yamagishi H, Nakazato E, Kimura Y, Shinjoh M, Yamagata T (2021) Clinical impact of bacteremia due to *Staphylococcus saprophyticus*. *Advances in Infectious Diseases* 11: 6-12. doi: 10.4236/aid.2021.111002.
 33. de Paiva-Santos W, de Sousa VS, Giambiagi-deMarval M (2018) Occurrence of virulence-associated genes among *Staphylococcus saprophyticus* isolated from different sources. *Microb Pathog* 119: 9-11. doi: 10.1016/j.micpath.2018.03.054.
 34. de Sousa VS, da-Silva AP de S, Sorenson L, Paschoal RP, Rabello RF, Pinheiro MS, Dos Santos LOF, Martins N, Botelho ACN, Picão RC, Fracalanza SEL, Riley LW, Sensabaugh G, Moreira BM (2017) *Staphylococcus saprophyticus* recovered from humans, food, and recreational waters in Rio de Janeiro, Brazil. *Int J Microbiol* 2017: 4287547. doi: 10.1155/2017/4287547.
 35. Lo DS, Shieh HH, Barreira ER, Ragazzi SLB, Gilio AE (2015) High frequency of *Staphylococcus saprophyticus* urinary tract infections among female adolescents. *Pediatr Infect Dis J* 34: 1023-1025. doi: 10.1097/INF.0000000000000780.
 36. Matarneh A, Ali GA, Goravey W (2021) Pyelonephritis-associated *Staphylococcus saprophyticus* bacteremia in an immunocompetent host: case report and review of the literature. *Clin Case Rep* 9: e05183. doi: 10.1002/ccr3.5183.
 37. Kline KA, Lewis AL (2016) Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Microbiology Spectrum*. doi: 10.1128/9781555817404.ch19.
 38. Taher I, Almaeen A, Aljourfi H, Bohassan E, Helmy A, El-Masry E, Saleh B, Aljaber N (2019) Surveillance of antibiotic resistance among uropathogens in Aljouf region northern Saudi Arabia. *Iran J Microbiol* 11: 468-477. doi: 10.18502/ijm.v11i6.2218.
 39. Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, Janet E, Victor LY. (2006) Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. *Clin Infect Dis* 42: 46-50. doi: 10.1086/498518.
 40. Karakonstantis S, Kalemaki D (2018) Evaluation and management of *Staphylococcus aureus* bacteriuria: an updated review. *Infection* 46: 293-301. doi: 10.1007/s15010-017-1100-6.
 41. Grillo S, Cuervo G, Carratalà J, Grau I, Llaberia M, Aguado JM, Lopez-Cortés LE, Lalueza A, Sanjuan R, Sanchez-Batanero A, Ardanuy C, García-Somoza D, Tebé C, Pujol M (2020) Characteristics and outcomes of *Staphylococcus aureus* bloodstream infection originating from the urinary tract: a multicenter cohort study. *Open Forum Infect Dis* 7: ofaa216. doi: 10.1093/ofid/ofaa216.
 42. Looney AT, Redmond EJ, Davey NM, Daly PJ, Troy C, Daly PJ, Troy C, Carey BF, Cullen IM (2017) Methicillin-resistant *Staphylococcus aureus* as a uropathogen in an Irish setting. *Medicine* 96: e4635. doi: 10.1097/MD.0000000000004635.
 43. Barros EM, Ceotto H, Bastos MCF, Santos KRN dos, Giambiagi-deMarval M (2012) *Staphylococcus haemolyticus* as an important hospital pathogen and carrier of methicillin resistance genes. *J Clin Microbiol* 50. doi: 10.1128/JCM.05563-11.
 44. Serpersu EH, Norris AL (2012) Chapter 5 - Effect of protein dynamics and solvent in ligand recognition by promiscuous aminoglycoside-modifying enzymes. In Horton D, editor. *Advances in carbohydrate chemistry and biochemistry* 67: 221-248. doi: 10.1016/B978-0-12-396527-1.00005-X.

45. Krause KM, Serio AW, Kane TR, Connolly LE (2016) Aminoglycosides: an overview. *Cold Spring Harb Perspect Med* 6: a027029. doi: 10.1101/cshperspect.a027029.
46. Haque MH, Miah ML, Sarker S, Shamsuzzaman M, Shiddiky MJ (2020) Molecular characterization and antibiogram profiling of multidrug resistant *Staphylococcus haemolyticus* isolated from patients with urinary tract infection in Bangladesh. *J Bacteriol Mycol* 8: 1-8. doi: 10.26420/jbacteriolmycol.2021.1166.
47. Farid A, Naz I, Ashraf A, Ali A, Rehman A-U, Sarwar Y, Haque A (2015) Molecular detection of antimicrobial resistance in local isolates of *Staphylococcus epidermidis* from urinary tract infections in Faisalabad region of Pakistan. *EXCLI J* 14: 697-705.
48. Gaillard T, Dormoi J, Madamet M, Pradines B (2016) Macrolides and associated antibiotics based on similar mechanism of action like lincosamides in malaria. *Malar J* 15: 85. doi: 10.1186/s12936-016-1114-z.
49. Aronson JK, editor (2016) *Macrolide antibiotics. Meyler's side effects of drugs* (Sixteenth edition). Oxford: Elsevier. 710-725. doi: 10.1016/B978-0-444-53717-1.01009-X.
50. Simon-Oke IA, Odeyemi O, Afolabi OJ (2019) Incidence of urinary tract infections and antimicrobial susceptibility pattern among pregnant women in Akure, Nigeria. *Scientific African* 6: e00151. doi: 10.1016/j.sciaf.2019.e00151.
51. Windahl U, Holst BS, Nyman A, Grönlund U, Bengtsson B (2014) Characterisation of bacterial growth and antimicrobial susceptibility patterns in canine urinary tract infections. *BMC Vet Res* 10: 217. doi: 10.1186/s12917-014-0217-4.
52. Daneman N, Chateau D, Dahl M, Zhang J, Fisher A, Sketris IS, Quail J, Marra F, Ernst P, Bugden S. (2020) Fluoroquinolone use for uncomplicated urinary tract infections in women: a retrospective cohort study. *Clin Microbiol Infect* 26: 613-618. doi: 10.1016/j.cmi.2019.10.016.
53. Rosana Y, Oeviyanti D, Karuniawati A, Akhmad SRP (2019) In vitro resistance pattern of urinary tract infections-causing bacteria to ampicillin and ciprofloxacin. *Obstet Gynecol Int J* 10: 372-376. doi: 10.15406/ogij.2019.10.00469.
54. Singh D, Goel D, Chand AE (2019) Prevalence of MRSA among *Staphylococcus aureus* isolated from patients of urinary tract infection along with its antibiogram. *IJMSCR* 2: 364-370.

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